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1 Please give the title of the invention: Novel Composition

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☐ **First or only applicant**

2a If you are applying as a corporate body please give:

Corporate Name: SmithKline Beecham Biologicals s.a.

Country *(and State of incorporation, if appropriate)*: Belgium

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## ❹ Reference number

4. Agent's or applicant's reference number *(if applicable)* KLP/RH B45197

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# Novel Composition

This invention relates to novel vaccine formulations, methods for preparing them and their use in therapy. In particular the present invention relates to combination vaccines for administration to adolescents.

Papillomaviruses are small DNA tumour viruses, which are highly species specific. So far, over 70 individual human papillomavirus (HPV) genotypes have been described. HPVs are generally specific either for the skin (e.g. HPV-1 and -2) or mucosal surfaces (e.g. HPV-6 and -11) and usually cause benign tumours (warts) that persist for several months or years. Such benign tumours may be distressing for the individuals concerned but tend not to be life threatening, with a few exceptions.

Some HPVs are also associated with cancers. The strongest positive association between an HPV and human cancer is that which exists between HPV-16 and HPV-18 and cervical carcinoma. Cervical cancer is the most common malignancy in developing countries, with about 500,000 new cases occurring in the world each year. It is now technically feasible to actively combat primary HPV-16 infections, and even established HPV-16-containing cancers, using vaccines. For a review on the prospects for prophylactic and therapeutic vaccination against HPV-16 see Cason J., Clin. Immunother. 1994; 1(4) 293-306 and Hagenesee M.E., Infections in Medicine 1997 14(7) 555-556,559-564.

Today, the different types of HPVs have been isolated and characterised with the help of cloning systems in bacteria and more recently by PCR amplification. The molecular organisation of the HPV genomes has been defined on a comparative basis with that of the well characterised bovine papillomavirus type 1 (BPV1).

Other HPV serotypes of particular interest are 31, 33 and 45.

carcinoma in situ (CIS) which are themselves regarded as precursor lesions of invasive cervix carcinoma.

WO 96/19496 discloses variants of human papilloma virus E6 and E7 proteins, particularly fusion proteins of E6/E7 with a deletion in both the E6 and E7 proteins. These deletion fusion proteins are said to be immunogenic.

HPV L1 based vaccines are disclosed in WO94/00152, WO94/20137, WO93/02184 and WO94/05792. Such a vaccine can comprise the L1 antigen as a monomer, a capsomer or a virus like particle. Such particles may additionally comprise L2 proteins. L2 based vaccines are described for example in WO93/00436. Other HPV vaccines are based on the Early proteins, such as E7 or fusion proteins such as L2-E7.

Vaccines for the prophylaxis of hepatitis B infections, comprising one or more hepatitis B antigens, are well known. For example the vaccine Engerix-B (Trade Mark) from SmithKline Beecham Biologicals is used to prevent Hepatitis B. This vaccine comprises hepatitis B surface antigen (specifically the 226 amino acid S-antigen described in Harford et. al. in Postgraduate Medical Journal, 1987, 63 (Suppl. 2), p65-70) and is formulated using aluminium hydroxide as adjuvant.

There is a need for effective combination vaccines to prevent diseases to which adolescents are particularly prone.

The present invention provides a vaccine composition comprising:

(a) a hepatitis B viral (HBV) antigen; and

(b) a human papillomavirus (HPV) antigen

in combination with an adjuvant which is a preferential stimulator of TH1 cell response.

and not cause any side-effects. It is stated that aluminium gel may be used, in particular aluminium hydroxide gel and aluminium phosphate gel.

In a further aspect, the invention provides a vaccine composition comprising:

(a) a hepatitis B viral (HBV) antigen;

(b) a human papillomavirus (HPV) antigen; and

(c) a hepatitis A viral (HAV) antigen

in combination with an adjuvant which is a preferential stimulator of TH1 cell response.

Such a vaccine is of great benefit for administration to adolescents who may be particularly at risk of HBV, and/or HPV infection, and/or HAV infection.

An immune response may be broadly divided into two extreme catagories, being a humoral or cell mediated immune response (traditionally characterised by antibody and cellular effector mechanisms of protection respectively). These categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

Extreme TH1-type immune responses may be characterised by the generation of antigen specific, haplotype restricted cytotoxic T lymphocytes, and natural killer cell responses. In mice TH1-type responses are often characterised by the generation of antibodies of the IgG2a subtype, whilst in the human these correspond to IgG1 type antibodies. TH2-type immune responses are characterised by the generation of a broad range of immunoglobulin isotypes including in mice IgG1, IgA, and IgM.

It can be considered that the driving force behind the development of these two types of immune responses are cytokines. High levels of TH1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of TH2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem, Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in European Patent 0 689 454 B1 (SmithKline Beecham Biologicals SA).

Preferably, the particles of 3D-MPL are small enough to be sterile filtered through a 0.22micron membrane (as described in European Patent number 0 689 454). 3D-MPL will be present in the range of 10μg - 100μg preferably 25-50μg per dose wherein the antigen will typically be present in a range 2-50μg per dose.

Another preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with an carrier.

The method of production of QS21 is disclosed in US patent No. 5,057,540.

Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol have been shown to be successful TH1 stimulating adjuvants when formulated together with an antigen. Thus vaccine compositions which form part of the present invention may include a combination of QS21 and cholesterol.

Further adjuvants which are preferential stimulators of TH1 cell response include immunomodulatory oligonucleotides, for example unmethylated CpG sequences as disclosed in WO 96/02555.

Combinations of different TH1 stimulating adjuvants, such as those mentioned hereinabove, are also contemplated as providing an adjuvant which is a preferential stimulator of TH1 cell response. For example, QS21 can be formulated together with 3D-MPL. The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10

In one preferred embodiment the HPV antigen in the vaccine composition according to the invention comprises the major capsid protein L1 of HPV and optionally the L2 protein, particularly from HPV 16 and/or HPV 18. In this embodiment, the preferred form of the L1 protein is a truncated L1 protein. Preferably the L1 is in the form of a virus-like particle (VLP). The L1 protein may be fused to another HPV protein, in particular E7 to form an L1-E7 fusion. Chimeric VLPs comprising L1-E or L1-L2-E are particularly preferred.

In another preferred embodiment, the HPV antigen in the composition of the invention is derived from an E6 or E7 protein, in particular E6 or E7 linked to an immunological fusion partner having T cell epitopes.

In a preferred form of this embodiment of the invention, the immunological fusion partner is derived from protein D of *Heamophilus influenza* B. Preferably the protein D derivative comprises approximately the first 1/3 of the protein, in particular approximately the first N-terminal 100-110 amino acids.

Preferred fusion proteins in this embodiment of the invention comprise Protein D - E6 from HPV 16, Protein D - E7 from HPV 16 Protein D - E7 from HPV 18 and Protein D - E6 from HPV 18. The protein D part preferably comprises the first 1/3 of protein D.

In still another embodiment of the invention, the HPV antigen is in the form of an L2-E7 fusion, particularly from HPV 6 and/or HPV 11.

The proteins of the present invention preferably are expressed in E. coli. In a preferred embodiment the proteins are expressed with a Histidine tail comprising between 5 to 9 and preferably six Histidine residues. These are advantageous in aiding purification. The description of the manufacture of such proteins is fully described in co-pending UK patent application number GB 9717953.5.

The HBsAg may be adsorbed on aluminium phosphate as described in WO93/24148.

Preferably the hepatitis B (HBV) antigen used in the formulation of the invention is HBsAg S-antigen as used in the commercial product Engerix-B (Trade Mark; SmithKline Beecham Biologicals).

A vaccine comprising hepatitis B surface antigen in conjunction with 3D-MPL was described in European Patent Application 0 633 784.

Examples of antigens from additional pathogens which may be included in the compositions according to the invention are now described.

Epstein Barr Virus (EBV), a member of the herpesvirus group, causes infectious mononucleosis as a primary disease in humans. Predominantly it affects children or young adults. More than 90% of the average adult population is infected by EBV that persists for lifetime in peripheral B-lymphocytes. The virus is lifelong produced in the parotid gland and spread primarily by exchange of saliva from individuals who shed the virus. Children infected with EBV are largely asymptomatic or have very mild symptoms, while adolescents and adults who become infected develop typical infectious mononucleosis, characterised by fever, pharyngitis, and adenopathy. People who have been infected maintain anti-EBV antibodies for the remainder of their lives, and are thus immune to further infection.

In addition to its infectious qualities, EBV has been shown to transform lymphocytes into rapidly dividing cells and has therefore been implicated in several different lymphomas, including African Burkitt's lymphoma (BL). EBV may also be involved in causing nasopharyngeal carcinoma (NPC). Worldwide it is estimated that 80,000 cases of nasopharyngeal carcinoma occur and it is more prevalent in ethnic Chinese populations. Infectious mononucleosis is a consequence of primary infection by EBV. It is not a life-threatening disease if additional risk factors are absent.

Both HSV-1 and HSV-2 virus have a number of glycoprotein components located on the surface of the virus. These are known as gB, gC, gD and gE etc.

When an HSV antigen is included in the composition of the invention this is preferably derived from HSV-2, typically glycoprotein D. Glycoprotein D is located on the viral membrane, and is also found in the cytoplasm of infected cells (Eisenberg R.J. et al; J of Virol 1980, 35, 428-435). It comprises 393 amino acids including a signal peptide and has a molecular weight of approximately 60 kD. Of all the HSV envelope glycoproteins this is probably the best characterised (Cohen et al; J. of Virology, 60, 157-166). In vivo it is known to play a central role in viral attachment to cell membranes. Moreover, glycoprotein D has been shown to be able to elicit neutralising antibodies in vivo (Eing et al J. Med. Virology 127: 59-65). However, latent HSV-2 virus can still be reactivated and induce recurrence of the disease despite the presence of high neutralising antibodies titre in the patients sera.

In one embodiment of the invention there is present a truncated HSV-2 glycoprotein D of 308 amino acids which comprises amino acids 1 through 306 naturally occurring glycoprotein with the addition Asparagine and Glutamine at the C terminal end of the truncated protein devoid of its membrane anchor region. This form of the protein includes the signal peptide which is cleaved to yield a mature 283 amino acid protein. The production of such a protein in Chinese Hamster ovary cells has been described in Genentech's European patent EP-B-139 417.

The recombinant mature HSV-2 glycoprotein D truncate is preferably used in the vaccine formulations of the present invention and is designated rgD2t.

A combination of this HSV-2 antigen in combination with the adjuvant 3D-MPL has been described in WO 92/16231.

In a preferred aspect the vaccine composition of the invention additionally comprises a Varicella Zoster viral antigen (VZV antigen). Suitable antigens of VZV for

In one preferred aspect the vaccine composition of the invention additionally comprises either a VZV antigen or an HCMV antigen combined with a *Toxoplasma gondii* antigen, in particular those antigens described above.

In a preferred aspect the vaccine composition of the invention is a multivalent vaccine, for example a tetra- or pentavalent vaccine.

The formulations of the present invention are very effective in inducing protective immunity, even with very low doses of antigen (e.g. as low as 5μg rgD2t).

They provide excellent protection against primary infection and stimulate, advantageously both specific humoral (neutralising antibodies) and also effector cell mediated (DTH) immune responses.

The present invention in a further aspect provides a vaccine formulation as herein described for use in medical therapy, particularly for use in the treatment or prophylaxis of human papillomavirus infections and hepatitis B virus infections.

The vaccine of the present invention will contain an immunoprotective quantity of the antigens and may be prepared by conventional techniques.

Vaccine preparation is generally described in Pharmaceutical Biotechnology, Vol.61 Vaccine Design - the subunit and adjuvant approach, edited by Powell and Newman, Plenurn Press, 1995. New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is

## CLAIMS

1. A vaccine composition comprising:

(a) a hepatitis B viral (HBV) antigen; and

(b) a human papillomavirus (HPV) antigen

in conjunction with an adjuvant which is a preferential stimulator of TH1 cell response.

2. A vaccine composition according to claim 1 which additionally comprises a carrier.

3. A vaccine composition according to claim 1 or claim 2 in which the preferential stimulator of TH1-cell response is selected from the group of adjuvants comprising: 3D-MPL, 3D-MPL wherein the size of the particles of 3D-MPL is preferably about or less than 100nm, QS21, a mixture of QS21 and cholesterol, and a CpG oligonucleotide.

4. A vaccine composition according to claim 3 in which the preferential stimulator of TH1-cell response is 3D-MPL.

5. A vaccine compostion according to any one of claims 1 to 4 in which the Hepatitis B antigen is hepatitis B surface antigen.

6. A vaccine composition according to any one of claims 1 to 5 which comprises at least one HPV antigen selected from the group consisting of L1, L2, E6 and E7, optionally in the form of a fusion protein or a truncate.

7. A vaccine composition according to any one of claims 1 to 6 in which an EBV antigen is additionally present.

8. A vaccine composition as defined in claim 7 in which the EBV antigen is gp 350.

19. A vaccine composition according to claim 18 in which the *Toxoplasma gondii* antigen is SAG1 or TG34.

20. A vaccine composition according to any one of claims 1 to 4 comprising HBsAg S antigen and L1, L2, E6, E7, protein D-E6, protein D-E7 or L2-E7 of HPV and optionally in addition one or more of HSV-2 gDt; EBVgp 350; VZVgpI; HAV HM-175 inactivated strain; gB685** or pp65 of HCMV and SAG1 or TG34 antigens of *Toxoplasma gondii*.